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☐ 1: Int J Biol Macromol. 1989 Jun;11(3):145-9. Links

Studies on the chemical constitution of cell wall lipo-oligosaccharide from Campylobacter coli Labet 227.

Adeyeye A, Ogunlesi M, Odugbemi T.

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The lipo-oligosaccharide (LOS) from Campylobacter coli Labet 227 was extracted by aqueous phenol. After delipidation and gel chromatography, two oligosaccharides were isolated. The higher molecular weight material OS (I) which was estimated to contain six to seven sugar units was found to contain glucose, galactose, 2-acetamido-2-deoxyglucose, 2-acetamido-2-deoxygalactose and heptose. Analysis of the partially methylated alditol acetates by g.c.-m.s. revealed the presence of terminal hexoses, a 1.3-linked hexose, a terminal heptose, a 1,2,3-linked heptose as well as smaller quantities of a 1,3,4-linked heptose. 1H-n.m.r. spectra showed signals corresponding to six anomeric protons. The signals which corresponded to the methyl protons of the acetamido side chain confirmed that the acetamido forms of both amino sugars were present in OS (I). The lower molecular weight material OS (II) which was estimated to contain four sugar units was found to contain glucose, 2-acetamido-2-deoxy-galactose and very small quantities of heptose. It thus appears that OS (I) and OS (II) are the core oligosaccharides elaborated by this micro-organism. The possibility of a heterogeneous core is thus presented. The fatty acids present in the LOS were mainly 3-hydroxytetradecanoic acid, n-hexadecanoic acid and trace amounts of n-tetradecanoic acid and n-octadecanoic acid.

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search notes

Prokaryotic glycosylation

Overview

Investigations into glycosylation systems in eukaryotic organisms have prevailed since the late 1930s, yet for many decades it was assumed that bacteria and archaea were devoid of this important protein modification (Messner, 2004). The discovery of surface layer (*S*-layer) glycoproteins in the Gram-negative halophile, *Halobacterium salinarium*, was the first such system to be found outside of the eukaryotic domain (Mescher and Strominger, 1976). These *S*-layer glycoproteins in archaea have the unique feature of assembling into two-dimensional crystalline arrays on the cell wall of halobacteria and are characterized by a variety of **glycans** and a diverse array of linkages to protein (Schaffer and Messner, 2004). Since this initial report of *S*-layer glycoproteins in halobacteria, several characterizations of similar glycosylated proteins in the bacterial domain have also surfaced (Messner, 1997). These glycoproteins are integrated into cell-surface appendages, such as pili and flagella (Power *et al.*, 2000; Messner, 2004). The pili of pathogenic bacteria, such as *Neisseria meningitides* (Stimson *et al.*, 1995) and *Neisseria gonorrhoeae* (Hegge *et al.*, 2004) contain *O*-linked **glycans** that involve unusual sugars such as 2,4-diacetamido-2,4,6-trideoxyhexose (DATDH) and pili of *Pseudomonas aeruginosa* contain analogs of *O*-linked pseudaminic acid (a nine-carbon sugar that resembles sialic acid) (Castric *et al.*, 2001). The flagella of Gram-negative bacteria such as *C. jejuni* (Thibault *et al.*, 2001) and *Helicobacter pylori* (Schirm *et al.*, 2003) have also been shown to include *O*-linked pseudaminic acid analogs.

The first system of *N*-linked protein glycosylation to be discovered in Gram-negative bacteria comes from *C. jejuni* (Szymanski, Logan *et al.*, 2003; Szymanski and Wren, 2005), a human-gut mucosal pathogen that is implicated in gastroenteritis. ***Campylobacter*** enteritis is characterized by acute abdominal pain and inflammatory diarrhea (Ketley, 1997), hence, understanding the pathogenicity of *C. jejuni* could potentially lead to better prevention and infection-control strategies. The sequencing of the *C. jejuni* genome, together with detailed genetic maps, has facilitated genetic characterization of various strains of this organism (Taylor *et al.*, 1992; Karlyshev *et al.*, 1998; Parkhill *et al.*, 2000; Fouts *et al.*, 2005).

In 1999, it was discovered that *C. jejuni* contains a gene locus that is involved in the biosynthesis of a number of highly immunogenic glycoproteins (Szymanski *et al.*, 1999). This cluster was termed the "*pgl* gene cluster" and contained the genes *pglA* to *pglG*, which demonstrate significant homology to enzymes involved in bacterial lipopolysaccharide (LPS) and capsular polysaccharide (CPS) biosynthesis. Mutagenesis of key residues in this cluster resulted in no discernible effect on CPS or LPS levels but caused a dramatic reduction in the immunoreactivity of numerous *C. jejuni* proteins, suggesting that these proteins functioned independently from the known LPS/CPS biosynthetic pathways.

The highly immunogenic ~~*C. jejuni*~~ proteins affected by mutations in the *pgl* gene cluster bind strongly to the soybean agglutinin (SBA) lectin, which is known to bind terminal GalNAc residues. This

observation allowed the identification of PEB3 and CgpA, two highly immunoreactive glycoproteins in *C. jejuni* (Linton *et al.*, 2002). The glycan attached to these proteins was not affected by ß-elimination, which generally removes *O*-linked **glycans**, thus suggesting a linkage *via* a glycosyl amide to an asparagine residue. Additionally, MS/MS collision-induced dissociation of the glycopeptide confirmed that the oligosaccharide was *N*-linked (Young *et al.*, 2002). Through the action of specific exoglycosidases, the oligosaccharide was shown to include one or more α-linked GalNAc residues (Linton *et al.*, 2002). The PEB3 glycoprotein was partially purified and analyzed by mass spectrometry to reveal a modification *via* an Asn-linked glycan with a mass of 1406 Da. Using nano-NMR techniques on the pronase-digested glycopeptides, the structure was determined to be the heptasaccharide, $GlcGalNAc_5Bac\beta1$,N-Asn where Bac is bacillosamine (2,4-diacetamido-2,4,6-trideoxyglucose) (Figure 6) (Young *et al.*, 2002). Furthermore, this heptasaccharide structure was shown to be conserved throughout all *C. jejuni* and *C. coli* strains examined (Szymanski, St Michael *et al.*, 2003).

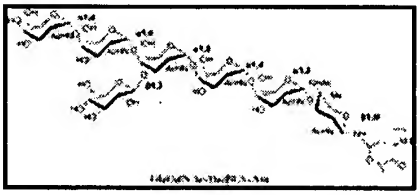

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Fig. 6. Structure of the heptasaccharide transferred to protein in *C. jejuni N*-linked glycosylation.

The *C. jejuni* heptasaccharide is structurally very different from the tetradecasaccharide transferred in eukaryotic *N*-linked glycosylation. Bacteria utilize a wide variety of amino- and deoxy-sugars that are not found in eukaryotic systems (Maki and Renkonen, 2004). This feature is exemplified by the *N*-linked glycan in *C. jejuni* that incorporates bacillosamine, a diacetamido-trideoxy-sugar found in several bacterial strains such as *Neisseria*. The first bacillosamine derivative was originally discovered in *Bacillus subtilis* (Sharon and Jeanloz, 1960), and since then several syntheses of bacillosamine have been reported (Liav *et al.*, 1973; Bundle and Josephson, 1980). Recently a synthetic route to undecaprenyl-pyrophosphate-linked bacillosamine (Und-PP-Bac) was described and this synthetic compound was utilized as a tool to investigate the enzymes in the *pgl* gene cluster *in vitro* (Weerapana *et al.*, 2005).

The Pgl pathway

Computational analysis of the *pgl* gene cluster (Figure 7) suggested that the locus encodes five putative glycosyltransferases (PglA, PglC, PglH, PglI, and PglJ), and three enzymes involved in sugar biosynthesis (PglD, PglE, and PglF). The PglB protein demonstrates significant homology to the Stt3p subunit of the yeast OT complex and WlaB is a putative ATP-binding cassette (ABC) transport protein (Linton *et al.*, 2002). The *gne* gene encodes a bifunctional UDP-Glc/GlcNAc 4-epimerase that converts UDP-Glc and UDP-GlcNAc to UDP-Gal and UDP-GalNAc. This gene is not exclusively involved in *N*-

linked glycosylation, and is also required for LPS and CPS biosynthesis (Bernatchez *et al.*, 2005).

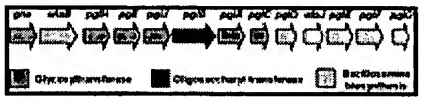

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Fig. 7. The *pgl* gene cluster from *C. jejuni*.

This *pgl* gene cluster in *C. jejuni* displays significant homology to a cluster found in the genome of *N. meningitides*, which is known to be responsible for the *O*-linked glycosylation of pilin (Figure 8). Pilin glycosylation involves an *O*-modified serine with a Gal-ß1,3-Gal-α1,3-DATDH modification (Stimson *et al.*, 1995). The stereochemistry of the DATDH sugar in pilin glycosylation has not been unambiguously determined, but is most likely to be bacillosamine. Bioinformatic analysis of the *pgl* gene cluster in *C. jejuni* was greatly facilitated by the fact that several homologous genes in the *N. meningitides* cluster were already functionally annotated (Power *et al.*, 2000). The genes involved in pilin glycosylation in *N. meningitides* are also denoted "*pgl*" similar to the *C. jejuni* gene cluster; however, the two classification systems are completely independent. Analogs of the sugar modifying enzymes, PglF, PglE, and PglD, are present in the *N. meningitides* cluster (Nm PglD, PglC, and PglB) and are attributed to the biosynthesis of bacillosamine. Recently, the PglF and PglE proteins from ***Campylobacter*** were studied *in vitro* and annotated as a dehydratase and amino transferase, respectively (Schoenhofen *et al.*, 2006). Nm *pglB* encodes a bi-functional protein demonstrating both glycosyltransferase and acetyltransferase activity. The *pglC* gene in *C. jejuni* encodes a protein that is homologous to the *N*-terminal portion of the Nm PglB protein and is thought to be responsible for transferring the first sugar phosphate onto a polyisoprene-phosphate carrier (Power *et al.*, 2000). The *pglA* gene in *C. jejuni* is homologous to Nm *pglA*, which is responsible for the Galα1,3-Bac linkage. The other putative glycosyltransferase genes in *C. jejuni* are *pglH*, *pglI*, and *pglJ*, but the bioinformatics data are insufficient to assign GalNAcα1,4- or Glcß1,3-transferase function to these genes (Young *et al.*, 2002). There is no ortholog of the *C. jejuni* PglB protein in *N. meningitides*.

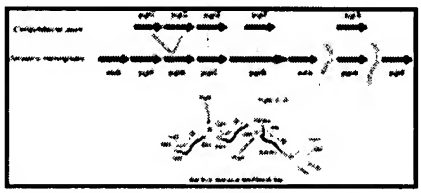

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Fig. 8. The pilin glycosylation locus of *N. meningitides* and its comparison to the *C. jejuni pgl* gene cluster.

In critical work by Aebi and coworkers, the *pgl* gene cluster was functionally transferred to *E. coli*, and two *C. jejuni* periplasmic proteins, AcrA and PEB3, were shown to be glycosylated in this modified *E. coli* system (Wacker *et al.*, 2002). This suggests that the *pgl* cluster contains all of the genes necessary for the biosynthesis of the polyisoprenyl-pyrophosphate-linked heptasaccharide and its eventual transfer to protein. It is postulated that the prokaryotic oligosaccharide is assembled onto a polyisoprenyl-pyrophosphate in a manner similar to the assembly of dolichyl-pyrophosphate-linked oligosaccharide in eukaryotes. The polyisoprene used is undecaprenol (also known as bactoprenol), and contains 11 isoprene units, where the α-isoprene unit is unsaturated, in contrast to the α-saturated nature of the corresponding unit in the dolichols (Wacker *et al.*, 2002). Analysis of ***Campylobacter*** isolates using the SBA lectin, which binds GalNAc residues, resulted in the isolation of up to 38 proteins that were identified as potentially containing this *N*-linked glycan (Young *et al.*, 2002). These glycoproteins are predominantly annotated as periplasmic proteins, which suggest that the glycosylation machinery is specific for periplasmic substrates. An AcrA mutant that lacks the periplasmic signal sequence is not glycosylated, further supporting the identification of the periplasm as the site of modification (Nita-Lazar *et al.*, 2005).

Through mutational studies of the *pgl* gene cluster in *E. coli*, the exact roles of various *pgl* genes were explored using structural analysis of the glycan transferred to protein (Linton *et al.*, 2005). As predicted by bioinformatics analysis, the *pglA*, *pglJ*, *pglH*, and *pglI* genes were shown to encode specific glycosyltransferases responsible for sequential addition of monosaccharides to form the ultimate heptasaccharide donor. The *pglA* mutant showed transfer of monosaccharide to protein, verifying the earlier observation that PglA transfers the α1,3-GalNAc to bacillosamine. The *pglJ* mutant showed transfer of disaccharide, suggesting that PglJ is responsible for the first α1,4-GalNAc linkage to afford the trisaccharide. The *pglH* mutant showed transfer of a trisaccharide to protein, suggesting a role for PglH in the transfer of the second α1,4-GalNAc sugar. Finally, the *pglI* mutant showed transfer of a linear hexasaccharide, suggesting its role as a glucosyltransferase, adding the final branching ß1,3-glucose residue (Figure 9). While this study provided crucial information on the role of several Pgl glycosyltransferases, it did not provide information on the identity of the transferases responsible for the addition of the two terminal α1,4-GalNAc residues. Therefore, the suggested scenarios were that PglH added all three terminal GalNAc residues or that PglH and PglJ acted alternately, adding two GalNAc residues each, to form the hexasaccharide.

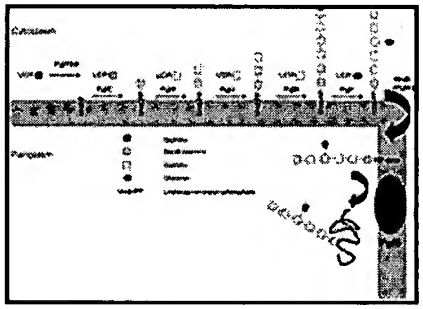

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Fig. 9. The Pgl pathway of *N*-linked protein glycosylation in *C. jejuni*.

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To further corroborate the mutagenesis data, the role of each of the Pgl enzymes was unambiguously validated through *in vitro* biochemical analysis, using chemically synthesized Und-PP-Bac and purified Pgl glycosyltransferases (Glover, Weerapana, Numao *et al.*, 2005; Weerapana *et al.*, 2005). These data provided further evidence to support the bioinformatics and mutational analyses above and also demonstrated that PglH is a sugar polymerase, adding three α1,4-linked GalNAc residues to the undecaprenyl-pyrophosphate-linked trisaccharide. Reconstitution of the sequence of enzymatic steps *in vitro* also provided valuable insight into how these enzymes function together at a membrane interface (Glover, Weerapana, and Imperiali, 2005).

PglB: the OT of C. jejuni

The *pglB* gene shares significant homology with the *STT3* gene that codes for the largest subunit of the yeast, *S. cerevisiae*, OT complex (Wacker *et al.*, 2002; Yan and Lennarz, 2002). PglB contains a highly conserved amino acid motif WWDYG that is present in all putative OT homologs. This conserved sequence is located on the hydrophilic *C*-terminal portion of PglB. In a *pglB* mutant strain, PEB3 and AcrA, both known glycoproteins from *C. jejuni*, were found to be unglycosylated (Wacker *et al.*, 2002, Young *et al.*, 2002). When functionally reconstituted in *E. coli*, the *pgl* cluster containing a mutation in the ^{457}WWDYG^{462} motif of PglB (W458A, D459A) resulted in unglycosylated protein (Wacker *et al.*, 2002; Nita-Lazar *et al.*, 2005). These results suggest the direct involvement of PglB in the glycosylation process, whereby PglB facilitates the transfer of the heptasaccharide onto the side chain of asparagine.

There are significant similarities between the Pgl pathway and the biosynthesis of the *O*-antigen LPS, where sequential addition of **glycans** results in an isoprenyl pyrophosphate-bound oligosaccharide that is transferred to the Lipid A core (Raetz and Whitfield, 2002). When the *O*-antigen ligase in *E. coli* was replaced with PglB, various *O*-antigen **glycans** were transferred to acceptor proteins (Figure 10) (Feldman *et al.*, 2005). This behavior illustrates the substrate flexibility of PglB, which can accept a diverse array of undecaprenyl-linked oligosaccharide substrates.

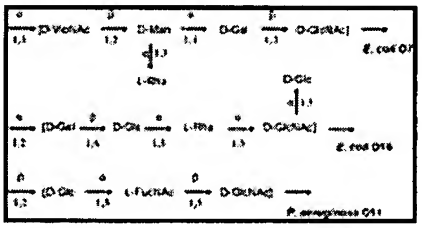

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Fig. 10. Diverse *O*-antigen **glycans** transferred to protein by PglB.

All of the glycosylated proteins identified in *C. jejuni* were shown to contain the Asn-Xaa-Ser/Thr sequon (Linton *et al.*, 2002). Other proteins in the genome also contain this sequon but do not appear to be glycosylated. Hence, similar to the eukaryotic system, it appears that the Asn-Xaa-Ser/Thr sequon is a necessary but not absolute determinant of glycosylation. Detailed investigation of the glycosylation sequon illustrated that, similar to the eukaryotic process, proline is not accepted as the Xaa amino acid, hence indicating the importance of peptide conformation in the glycosylation process (Nita-Lazar *et al.*, 2005). *In vitro* studies using an *E. coli* cell membrane fraction expressing PglB showed that, similar to the yeast OT system, PglB can accept a truncated peptide substrate (KDFNVSKA-NH_2) in place of a full-length protein. Initial studies indicate that the recognition sequence for PglB may require determinants in addition to the canonical tripeptide substrate (Bz-NLT-NHMe) for yeast OT (Glover, Weerapana, Numao *et al.*, 2005).

The glycosyl modifications synthesized by the *pgl* genes are highly immunogenic (Szymanski *et al.*, 1999). Mutations in *pglB* and *pglE* resulted in a significant reduction in adherence to, and invasion of, INT407 cells *in vitro*, and a reduced ability to colonize the intestinal tract of mice, suggesting a role for these *N*-linked **glycans** in ***Campylobacter*** virulence (Szymanski *et al.*, 2002). The immunogenic nature of *N*-linked **glycans** was further demonstrated by a *pglH* mutant strain that displayed reduced adherence to, and invasion of, human epithelial cells and affected the colonization of chicks (Karlyshev, Everest *et al.*, 2004). Recently it was demonstrated that the *N*-linked glycan in *C. jejuni* plays a direct role in complex protein assembly. VirB10 is an *N*-linked glycoprotein that is present in the type IV secretion system (T4SS) of *C. jejuni*. Lack of VirB10 glycosylation results in *C. jejuni* cells containing a competence defect due to lack of protein complexation (Larsen *et al.*, 2004). Interestingly, the closest homolog of the VirB10 glycoprotein is found in *Wolinella succinogenes*, which is the only other bacterium currently known to contain a putative *N*-linked glycosylation system similar to the *pgl* system.

Due to the essential role played by the *C. jejuni N*-**glycans** in bacterial adherence and pathogenicity, PglB and the Pgl pathway as a whole appear to be interesting potential targets for antibacterial therapeutics. The extensive work done on the synthesis of inhibitors for the eukaryotic OT system (Kellenberger *et al.*, 1997) can now be applied toward the design of potent inhibitors of PglB. The periplasmic location of PglB also makes it a much more accessible target than the eukaryotic OT complex that is located in the ER lumen.

Conclusion

There are several striking similarities as well as differences in the *N*-linked glycosylation processes of eukaryotic and prokaryotic systems. A summary of the characteristics of each of the systems is provided in Table I.

View this table: **Table I.** Comparison of yeast and bacterial *N*-glycosylation machinery

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The process of protein translocation and glycosylation is a well-characterized eukaryotic phenomenon. Significant work has been done on the role of the translocon, the signal peptidase and OT in this protein modification process. The exact machinery involved in bacterial *N*-linked glycosylation, however, is poorly defined. The glycosylation process is thought to occur in the periplasm of bacteria, which is the functional equivalent of the ER in eukaryotes. Currently, there is no conclusive evidence to demonstrate either the co-translational or post-translational nature of *N*-linked glycosylation in the bacterial periplasm. If the *C. jejuni* machinery functions post-translationally, on fully folded proteins, it could potentially be one of the most significant differences between the eukaryotic and prokaryotic processes of *N*-linked glycosylation.

The calnexin/calreticulin cycle exemplifies the intricate mechanisms by which eukaryotic cells maintain protein quality control to prevent the release of misfolded proteins into the extra-cellular milieu. Such a complex system of glycoprotein folding has not been demonstrated in the bacterial system, and the processes by which these organisms maintain glycoprotein quality is a prevailing question. Additionally, the glycan structures displayed on all *N*-linked glycoproteins of *C. jejuni* are identical, lacking the immense diversity of eukaryotic *N*-linked glycoproteins. This characteristic is due to the lack of the glycan trimming/elaboration steps that occur post-glycosylation in the Golgi of eukaryotic cells. Since bacterial cells lack the extensive compartmentalization present in eukaryotic cells, there is no functional equivalent of the Golgi, where such elaboration steps can take place.

The bacterial Pgl pathway shares striking similarities with the eukaryotic dolichol pathway. The oligosaccharide substrate for the OT is built up sequentially on a polyisoprenyl-pyrophosphate carrier (undecaprenol in *C. jejuni*, dolichol in *S. cerevisiae*) by a series of glycosyltransferases that utilize nucleotide-activated sugar donors or dolichol-phosphate-activated sugar donors. This sequence of biosynthetic transformations occurs in the periplasmic membrane of *C. jejuni*. Interestingly, in both systems, the glycan is built up to a heptasaccharide structure on the cytoplasmic face and then flipped to the other side of the membrane, either the ER lumen or the periplasm, by a flippase. The flippase in the *C. jejuni* system, WlaB, has been annotated to be an ABC transporter, whereas the Rft1p protein in the dolichol pathway is non-ATP dependent. In the dolichol pathway, this heptasaccharide is further elaborated to the tetradecasaccharide, whereas in *C. jejuni*, no further elaboration occurs. Both pathways contain at least one enzyme that catalyzes the transfer of multiple **glycans** (PglH in *C. jejuni*, Alg9 in yeast). One striking difference is that the *ALG* genes in yeast encode highly hydrophobic proteins, which all include at least one transmembrane domain. Although the Pgl glycosyltransferases function on similar isoprene-bound intermediates, they contain no predicted transmembrane domains. This renders the Pgl enzymes significantly more amenable to detailed biochemical analysis. Both pathways are examples of multistep enzymatic transformations that occur at a membrane interface. Studies devoted to understanding the interactions that occur amongst the Pgl glycosyltransferases at the membrane interface can provide clues on how the corresponding Alg enzymes maintain the fidelity of the dolichol pathway.

The OT complex in *S. cerevisiae* and PglB in *C. jejuni* both catalyze a similar reaction, the transfer of an oligosaccharide from a polyisoprenyl-pyrophosphate-linked glycan to an asparagine side chain. Yet, the *S. cerevisiae* system requires at least 8 proteins to efficiently catalyze this process, whereas the bacterial system appears to use only a single protein. In the yeast system, OT is required to interact with multiple other protein complexes such as the translocon and the signal peptidase for efficient co-translational glycosylation. It is hypothesized that some of the OT subunits play a role in these interactions. A predominant area of current research is to purify PglB to homogeneity in order to discern whether it is solely responsible for catalysis. Regardless, the simplicity of the PglB-mediated process provides us a great opportunity to investigate the mechanism of this intriguing enzymatic reaction in more depth.

The OT complex in *S. cerevisiae* shows a high degree of specificity with regards to the dolichyl-pyrophosphate-linked glycan substrate and accepts very few truncated and non-native structures (Karaoglu *et al.*, 2001; Tai and Imperiali, 2001). PglB, on the other hand, appears to display much greater glycan flexibility by accepting various *O*-antigen structures as well as **glycans** of varying length and structure (Feldman *et al.*, 2005; Glover, Weerapana, Numao *et al.*, 2005). The **glycans** are, however, limited to those containing a C-2 *N*-acetamido-group on the proximal sugar, which suggests a role of the *N*-acetamido group in the catalytic mechanism of both OT and PglB. This substrate promiscuity of PglB suggests great promise for the potential of using the bacterial glycosylation system in engineering humanized glycoproteins.

Glycosylated proteins in both the yeast and ***Campylobacter*** systems contain the Asn-Xaa-Ser/Thr consensus sequence. In both systems, glycosylation is abolished when the Xaa amino acid is proline, suggesting that local conformation plays an important role in the glycosylation reaction. Although the minimum recognition motif for the eukaryotic system is a simple -Asn-Xaa-Ser/Thr- tripeptide sequence, initial studies indicate that the aspartate residue in the -Asp-Xaa-Asn-Xaa-Ser/Thr- sequence found in ***Campylobacter*** glycoproteins is important for recognition by PglB.

Due to the similarities between the dolichol (Alg) pathway and the Pgl pathway, as well as the parallels between the Stt3p-catalyzed glycosylation with the PglB reaction, the eukaryotic and prokaryotic systems are greatly intertwined. Our knowledge accumulated over decades of research devoted to understanding eukaryotic *N*-linked glycosylation can now be applied to the recently discovered prokaryotic system. Hopefully, the reduced complexity of the *C. jejuni* glycosylation process will allow for detailed biochemical and biophysical characterization that is currently virtually impossible with the eukaryotic system. Hence, the knowledge that can be gained from understanding the prokaryotic process will be invaluable in shedding light on the mechanism and function of the eukaryotic glycosylation system.

Conflict of interest statement

None declared.

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▲ Prokaryotic glycosylation
▲ Conclusion
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▼ Acknowledgments
▼ References

Acknowledgments

The authors acknowledge the NIH (GM39334) for support of their research on eukaryotic and prokaryotic asparagine-linked glycosylation.

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▼ References

Abbreviations

CPS, capsular polysaccharide; DATDH, 2,4-diacetamido-2,4,6-trideoxyhexose; ER, endoplasmic reticulum; LPS, lipopolysaccharide; OT, oligosaccharyl transferase

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Eighthly, that antibodies prepared against .beta.-emulsan cross-react in an identical fashion with .alpha.-emulsan, apo-.alpha.-emulsan, apo-.beta.-emulsan, .psi.-emulsan and proemulsan, indicating that the emulsans and their deproteinized and partially deacylated derivatives have approximately the same polymer backbones, which are poly[D-galactosamine/aminouronic acid] polymers;

Detailed Description Text (63):
Each of the precipitates was dissolved in water and was then dialyzed at 2.degree.-5.degree. C. successively against distilled water, 0.05 N hydrochloric acid for 24 hours and double distilled water, following which each of the resulting solutions were freeze-dried. Over 99% of the emulsifying activity of the apo-.alpha.-emulsan was found in the two fractions which precipitated between 30% and 35% ammonium sulfate saturation. These two fractions contained similar specific activities and exhibited substantially the same chemical composition. In addition, both fractions were homogeneous when examined by immunodiffusion against antibodies prepared against .beta.-emulsan, each giving a single identical band upon Ouchterlony two-dimensional diffusion. Accordingly, the two fractions were combined for certain of the chemical and physical characterizations, the combined fractions when used being identified herein as "apo-.alpha.-emulsan-WA".

Detailed Description Text (105):
To immunologically characterize the Acinetobacter bioemulsifiers produced by Acinetobacter Sp. ATCC 31012, rabbits were injected with 1 mg of .beta.-emulsan in 1 ml complete Freund adjuvant. The rabbits were bled 11 to 14 days later, from which sera a crude immunoglobulin fraction was obtained by ammonium sulfate fractionation.

Detailed Description Text (106):
Antibodies prepared against .beta.-emulsan cross-react in an identical fashion with .alpha.-emulsan, apo-.alpha.-emulsan, apo-.beta.-emulsan, .psi.-emulsan (produced by mild base hydrolysis of .alpha.- or .beta.-emulsan) and proemulsan (produced by strong base hydrolysis of any of the foregoing), indicating that both Acinetobacter bioemulsifiers (.alpha.-emulsan and .beta.-emulsan) and their various deproteinated and deacylated derivatives have approximately the same polymer backbone, even though these classes of biopolymers are distinguishable by fatty acid ester content as well as by differences in the distributions of fatty acids, the .alpha.-emulsans containing a larger amount and greater proportion of 3-hydroxydodecanoic acid ester than the .beta.-emulsans.

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☐	L16	(GALNAC-ALPHA-1-P | GALNAC-ALPHA-1-PHOSPHATE | "GALNAC-ALPHA.1.FWDARW.3")!	3
☐	L15	(GALNAC-ALPHA1 | GALNAC-ALPHA | GALNAC-ALPHA-BZ | GALNAC-ALPHA-O-SER/THR-POLYPEPTIDE+UDP | GALNAC-ALPHA-O-SERINE | GALNAC-ALPHA-O-THREO)!	11
☐	L14	((EMULSAN)! same (antibodies or adjuvant or immun$ or immuniz$ or antibod$))	28
		DB=DWPI,JPAB,EPAB,USOC,USPT,PGPB; PLUR=YES; OP=OR	
☐	L13	(GALNAC4 | "GALNAC4SO.SUB.3")!	51
☐	L12	(GALNAC1-ALPHA-4MU)!	3
☐	L11	(GALNAC1)!	7
☐	L10	(GALNACS | GALNACP)!	10
☐	L9	"GALNACGLCNAC.BETA.1-3GALNAC"!	1
☐	L8	GALNACGAL!	8
☐	L7	("GALNACC.ALPHA")!	1
☐	L6	(GALNACA1-4 | "GALNACA.ALPHA" | "GALNACA.ALPHA-O-BN")!	2
☐	L5	(GALNACA1 | GALNACA1-GALNACB1-4)!	6
☐	L4	(GALNACALPHA1)!	3
☐	L3	(GALNACALPHA-SERINE | GALNACALPHA-THREONINE)!	1
☐	L2	(GALNACALPHA-SERINE | GALNACALPHA-THREONINE)!	1
☐	L1	(GALNACALPHA | GALNACALPHAL | GALNACALPHA1 | GALNAC | GALNACA)!	2261

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Identification of N-acetylgalactosamine-containing glycoproteins PEB3 and CgpA in Campylobacter jejuni.

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It was demonstrated recently that there is a system of general protein glycosylation in the human enteropathogen Campylobacter jejuni. To characterize such glycoproteins, we identified a lectin, Soybean agglutinin (SBA), which binds to multiple C. jejuni proteins on Western blots. Binding of lectin SBA was disrupted by mutagenesis of genes within the previously identified protein glycosylation locus. This lectin was used to purify putative glycoproteins selectively and, after sodium dodecyl sulphatepolyacrylamide gel electrophoresis (SDS-PAGE), Coomassie-stained bands were cut from the gels. The bands were digested with trypsin, and peptides were identified by mass spectrometry and database searching. A 28kDa band was identified as PEB3, a previously characterized immunogenic cell surface protein. Bands of 32 and 34kDa were both identified as a putative periplasmic protein encoded by the C. jejuni NCTC 11168 coding sequence Cj1670c. We have named this putative glycoprotein CgpA. We constructed insertional knockout mutants of both the peb3 and cgpA genes, and surface protein extracts from mutant and wild-type strains were analysed by one- and two-dimensional polyacrylamide gel electrophoresis (PAGE). In this way, we were able to identify the PEB3 protein as a 28 kDa SBA-reactive and immunoreactive glycoprotein. The cgpA gene encoded SBA-reactive and immunoreactive proteins of 32 and 34 kDa. By using specific exoglycosidases, we demonstrated that the SBA binding property of acid-glycine extractable C. jejuni glycoproteins, including PEB3 and CgpA, is a result of the presence of alpha-linked N-acetylgalactosamine residues. These data confirm the existence, and extend the boundaries, of the previously identified protein glycosylation locus of C. jejuni. Furthermore, we have identified two such glycoproteins, the first non-flagellin campylobacter glycoproteins to be identified, and demonstrated that their glycan components contain alpha-linked N-acetylgalactosamine residues.

Mesh-terms: Acetylgalactosamine, metabolism; Amino Acid Sequence; Bacterial Proteins, genetics; Bacterial Proteins, isolation & purification; Bacterial Proteins, metabolism; Campylobacter jejuni, genetics; Campylobacter jejuni, metabolism; Chromatography, Affinity, methods; Galactosaminidase, metabolism; Genes, Bacterial; Lectins, metabolism; Membrane Glycoproteins, genetics; Membrane Glycoproteins, isolation & purification; Membrane Glycoproteins, metabolism; Molecular Sequence Data; Mutagenesis; Plant Lectins; Soybean Proteins; Spectrum Analysis, Mass, methods; Support, Non-U.S. Gov't; beta-N-Acetylhexosaminidase, metabolism;